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THE INFLUENCE OF SIZE ON THE RADULA OF *LITTORARIA ANGULIFERA* (GASTROPODA: LITTORINIDAE)

Sónia C. S. Andrade & Vera N. Solferini

Departamento de Genética e Evolução, Instituto de Biologia, Universidade Estadual de Campinas (UNICAMP), C.P. 6109, CEP 13083-970, Campinas, São Paulo, Brazil; soniacsandrade@gmail.com

ABSTRACT

We evaluated the radula shape of *Littoraria angulifera* from mangroves and rocky shores on the Brazilian coast. We compared snails sampled in a mangrove and on a rocky shore located about 100 m apart, and also from locations hundreds of km apart. Smaller individuals sampled on rocky shores have elongated cusps, whereas larger individuals have subequal cusps, similar to those observed in mangrove specimens. Distinct factors in each environment might exert different effects on the radula shape of *L. angulifera*. The greater morphological diversity observed on the rocky shores might be due to the heterogeneity of algae composition and partitioning of resources, a hypothesis that can be tested in further studies.

Key words: *Littoraria angulifera*, mangroves, phenotypic variation, radula, rock substratum.

INTRODUCTION

Phenotypic variation in radulae has been studied in several littorinid species because of its intrinsic relationship with diet (Padilla, 1985, 1989, 1998; Padilla et al., 1996; Trussell, 2000; Ito et al., 2002). Differences in the morphology of this structure have been related to sexual dimorphism (Fujioka, 1985; Mutlu, 2004), diet (Padilla, 1998; Padilla et al., 1996), temporal and spatial variations in substrata (Reid & Mak, 1999; Ito et al., 2002), size (Isarankura & Runham, 1968), and age (Nybakken, 1990; Kawamura et al., 2001). The radula of littorinids is of the taenioglossate type, characterized by a central rachidian, surrounded by a pair of lateral teeth, a pair of inner marginal teeth, and a pair of outer marginal teeth. The functional significance of the form of radular teeth is still unclear, but it is probably closely associated with feeding habits and, consequently, with the environment (Padilla, 1985, 2004; Padilla et al., 1996; Reid, 1996). The radula has been used as an important source of characters in gastropod systematics, both for taxonomy and for phylogenetic reconstruction. Evaluation of intraspecific variation in radular form can, in some cases, bring about an understanding of its cause.

Littoraria Griffith & Pidgeon, 1834, is a genus with 39 known species that occur in mangroves, salt marshes, and rocky shores (Reid 1986, 1999, 2001). Two species are found along the Brazilian coast: *L. flava* (King & Broderip, 1832), usually restricted to rocky shores near freshwater, but also found in mangroves (Reid & Mak, 1999), and *L. angulifera* (Lamarck, 1822), which occurs in mangroves and, occasionally, on nearby rocky shores (Gallagher & Reid, 1974). Kohlmeyer & Bebout (1986) suggested that the diet of *L. angulifera* was based on fungus-infested plants and fungi. Reid & Mak (1999) reported differences in the radular form of this species collected on rocky shores and on mangroves that are probably related to environmental conditions. In this study, we compared radula from *L. angulifera* individuals collected on mangroves and rocky shores in different locations, trying to assess if such variation could be observed in Brazilian samples.

MATERIALS AND METHODS

Part of the samples was conducted on Dura beach (23°29'S, 45°10'E), a sheltered location in the Escuro River estuary at Ubatuba. At this location, both environments are present,

separated by about 100 m – a mangrove along the estuary and a salt-water rocky shore. Snails were collected from both environments. The mangrove vegetation at the Escuro River consisted mainly of three species, the marsh grass *Spartina* sp., and *Avicennia schaueriana* and *Laguncularia racemosa*, very common shrubs in the mangroves of southeastern Brazil. The sampling was done on February 20, 2003. Three samples of ten individuals were collected: on the rocky shore, on *Spartina* blades, and on the stems and aerial roots of *L. racemosa* and *A. schaueriana*.

Radulae from all 30 individuals were studied, but only 21 resulted in good images for electronic microscopy and were actually photographed. In the laboratory, the snails were immersed in 7.5% MgCl solution and then fixed in 70% ethanol. The shell heights (maximum dimension parallel to the axis of coiling) were measured to the nearest 0.01 mm with a digital caliper. To isolate the radula, the snails were kept in 7.5% KOH at 50°C until the soft body was completely dissolved (10–20 min), followed by gentle cleaning with a fine paintbrush and rinsing with distilled water. (In a previous study, heating did not cause any change to the radulae, compared with non-heated samples.) The radulae were mounted flat on aluminum stubs and held in place with dual adhesive tape. The structures were coated with gold and examined by scanning electron microscopy (SEM). Only fully formed and unworn teeth from the central third of the radula ribbon were examined. The sample size used for SEM analysis and the range of shell height are shown in Table 1. Photographs were taken in two standard orientations: at 45° from the front end of the radula and at 45° from the side of the radula, to show shape of tooth cusps and relief.

Radulae from periwinkles collected on rock and mangrove substrates from distant locations were also compared. Eighteen individuals collected on Mangaratiba mangrove shrubs (southeastern coast; 22°56’S, 44°4’E; September 1998) and 13 specimens from the rocky shore in Santa Barbara Island, Abrolhos Archipelago, (northeastern coast; 18°21’S, 38°36’E; October 1998) were also examined using a light microscope.

The description of the radula characters was based on Reid & Mak (1999); in our study, the major cusps are referring only to the lateral and inner marginal teeth. The counting of cusps was restricted to 4–20 rows for each individual based on photos of SEM or as seen in a light microscope. The number of cusps was counted on all visible teeth on both left and right sides of each radula. The characterization of the form of the major cusp (relative to the smallest cusps) on lateral and inner

TABLE 1. Specimens of *Littoraria angulifera* examined with SEM and light microscopy. The form of the major cusps of the lateral and inner marginal teeth and the number of cusps are shown for each sampling site (see text, Fig. 1 for explanations). The number of cusps considered was the observed on the 4–20 rows analyzed in the third central part of the radulae. The sample size is shown in parentheses. SH = range of shell height (mm). As “shrubs” we indicated the samples collected on *L. racemosa* and/or *A. schaueriana*.

Samples	SH	Form of major cusps	Lateral teeth	Inner marginal teeth	Outer marginal teeth
Dura Beach					
Rocky shore, stone (5)	6.7–9.4	elongate	5	4	4, 5
Rocky shore, stone (3)	14.6–18.0	subequal	5	4	5, 6
Mangrove, *Spartina* (4)	6.7–10.1	subequal	5, 6	4	6
Mangrove, *Spartina* (3)	10.9–12.6	subequal	5, 6	4	6
Mangrove, shrubs (6)	11.1–17.1	subequal	5, 6	4	6
Mangaratiba					
Mangrove, shrubs (11)	5.9–9.7	subequal	5, 6	4	5, 6
Mangrove, shrubs (1)	6.4	elongate	5	4	5
Mangrove, shrubs (6)	10.3–22.0	subequal	5, 6	4	6
Santa Barbara Island					
Rocky shore, stone (9)	5.2–10.7	elongate	5	4	4
Rocky shore, stone (4)	13.0–22.8	subequal	5, 6	4	4, 5

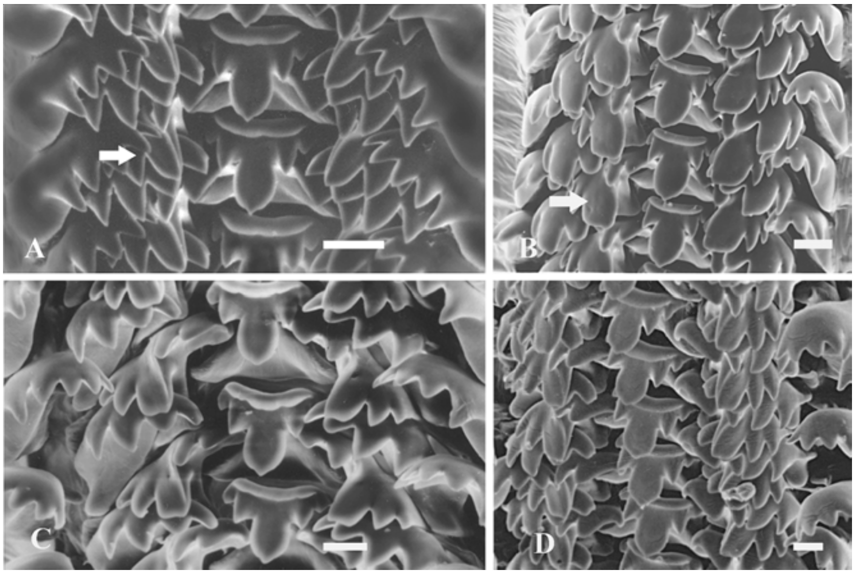

FIG. 1. Radulae of *Littoraria angulifera* samples. A: Rocky shore (shell height = 14.6 mm), lateral teeth subequal (arrow), at 45° from front; B: Rocky shore (shell height = 6.7 mm), lateral tooth elongate (arrow), at 45° from side; C: Mangrove, on *Spartina* (shell height = 10.9 mm), at 45° from front; D: Mangrove, on shrubs (shell height = 17.0 mm), at 45° from side. Scale bars = 10 μm.

marginal teeth was assigned in the following categories: subequal, characterized by sharper, more uniform cusps (e.g., Fig. 1A), and elongate, characterized by larger, blunt cusps (e.g., Fig. 1B).

To evaluate the influence of size on the shape of cusps, we performed a logistic regression. The dependent variable was the shape, codified as subequal (0) or elongate (1). Here, the maximum-likelihood method was used to fit a regression line to a logit-transformed data, since the dependent variable was binomially distributed (Sokal & Rohlf, 1995: 767–778).

RESULTS

The radulae in all analyzed individuals had a large hood in the all environments with similar number of cusps in the outer marginal teeth in both mangroves and rocky shores (Table 1, Fig. 1). Among individuals, there was variation in the number of cusps in the lateral and outer marginal teeth, but with no evident pattern (Table 1). On the other hand, there was no intra-individual variation in the number of cusps in the analyzed rows.

Littoraria angulifera showed little differentiation in the shape of the major cusps between environments (Figs. 1A, C), even among the different locations. Among specimens from the rocky shore in Dura and Santa Barbara, individuals with different shell heights showed variation in the cusp shape on the lateral teeth (Figs. 1A, B). All individuals with a shell height equal to or less than 10 mm had blunter, larger cusps in lateral and inner marginal teeth than found on specimens with greater shell height (Table 1, Fig. 1B). In mangroves, this variation between small and large periwinkles was not observed (Figs. 1C, D), except for one specimen at Mangaratiba (Table 1).

Shell length had a significant association with cusp shape for snails from Dura and Santa Barbara rocky shores (logistic regression analysis: χ^2 = 10.58 and 15.9, d.f. = 1, p = 0.0011 and 0.00007, respectively). There was no similar relationship for individuals from Mangaratiba (χ^2 = 1.99, d.f. = 1, p = 0.15). There were no differences in the shape of the radulae among snails collected on shrubs and *Spartina* (Figs. 1C, D). Thus, the regression analysis was not performed for these individuals.

DISCUSSION

Reid & Mak (1999) reported differences in the radular form in *Littoraria* species collected on rock and plant substrata: individuals from the rocky shore had a markedly enlarged major cusp on each of the five central teeth and fewer cusps on the outer marginal teeth, compared with radulae of conspecifics from mangroves. We observed no difference in the shape of the radula between large snails from these two environments among all locations, in contrast to the observations of Reid & Mak (1999). The samples used in their study were collected in different places (Belize and Florida; D. G. Reid, personal communication), which could explain the variation observed. Our samples were collected at geographical distances ranging from 240 km (between Dura and Mangaratiba) to 1,160 km (between Dura and Santa Barbara). There was also temporal variation among samples. Even though our samples were collected from very distant locations, cusp shape varied less than those analyzed by these authors. There was only a slight difference between the mangrove and rocky shore samples in the number of lateral and outer marginal cusps (Table 1). Thus, it is possible that these differing findings might reflect interpopulational genetic variation, assuming that radular variation is a heritable character.

On the rocky shore, the cusps of the lateral teeth varied in shape depending on the size of the snails; the smallest individuals had elongated cusps. This could represent a normal ontogenetic process, as observed in *Conus* (Nybakken, 1990), but we do not know if the small periwinkles sampled were young adults or juveniles. Only one small individual in the Mangaratiba mangrove had elongated cusps, which could be explained by a low frequency of individuals bearing this cusp shape. In Mangaratiba, mangrove and rock substrata are very close to each other (50–80 m), and this individual could have moved to the mangroves immediately before sampling. Possible explanations of the variation observed in the rocky shore are: (i) individuals of different sizes from rock substrata may have distinct diets and may partition resources; (ii) this pattern results from an ontogenetic effect, which is dependent on environmental characteristics; (iii) distinct factors in each environment may have exerted different selective effects on radula shape in *L. angulifera*; and (iv) an interaction among these factors could have contributed to the divergent effect seen in these two environments.

Merkt & Ellison (1998) noticed that several morphological traits of *L. angulifera* also vary in response to fine-scale environmental conditions. These authors observed that morphological variation in some traits, such as shell shape, sculptural, and genital morphology, could be ascribed to habitat characteristics and environmental heterogeneity.

In a previous study at Dura Beach, we observed that *L. flava*, which inhabits the same environments as *L. angulifera*, showed variation in radular tooth shape and length (Andrade & Solferini, 2006). Snails collected on mangroves had elongate teeth, whereas specimens from mangroves had sharper, subequal cusps. This variation is expected in snails in general, because sharper and pointed teeth as observed in mangroves and rocky shore large individuals have less contact with the surface and are more effective at piercing and tearing fleshy algae, whereas elongated teeth seem to be more effective for rasping and removing loose material from surfaces or broad excavations of brittle materials, such as calcified algae (Padilla, 2004). However, no influence of size was observed in individuals of *L. flava* sampled in rocky shore and mangroves (Andrade & Solferini, 2006). This finding indicated that the environments might exert different effects on the radulae of this species. The diet of *L. flava* has not been investigated, whereas *Littoraria angulifera* has been described as a generalist, because its diet seems to be very diverse (Kohlmeyer & Bebout, 1986). Feeding choice in gastropods is not a simple issue and depends of several factors, such as local abundance, chemical defenses, nutritional income, among others (Padilla, 1985, 1989). Thus, it is likely that the diverse flora found on the rock substrata (and maybe also the mangroves) allow *L. angulifera* to partition resources. In this species, the greater heterogeneity of the rocky shore, with the availability of different substrata, compared with mangroves, could have resulted in a greater morphological diversity. More comprehensive experiments isolating ontogeny and environmental effects on littorinids are necessary to help solve this question. Additional experimental work will be important for understanding the processes leading to the definition of the morphological traits in littorinids.

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